April 26, 2001

VETERINARY SERVICES MEMORANDUM NO. 800.99

Subject: Guidelines for Using *In Vitro* Relative Potency Tests to Determine the Antigen Content of Inactivated Bovine Rhinotracheitis Vaccine

To: Veterinary Biologics Licensees, Permittees, and Applicants
Directors, Center for Veterinary Biologics

I. PURPOSE

This memorandum provides guidelines for *in vitro* potency tests for serial release of bulk or final container samples of inactivated bovine rhinotracheitis vaccine. The guidelines outlined below include recommended procedures for the qualification and requalification of *in vitro* reference preparations as provided in Title 9, Code of Federal Regulations, Part 113, Section 113.8 (9 CFR 113.8).

II. BACKGROUND

APHIS has published the Standard Requirement for Bovine Rhinotracheitis Vaccine in 9 CFR 113.216. The regulation specifies that bulk or final container samples of the completed product must be tested for potency using a host animal serum neutralization test in cell culture or a virus challenge test of host animals if the results of the serum neutralization test are unsatisfactory. APHIS has granted an exemption to this requirement under 9 CFR 113.4 for firms using validated tests that determine potency by comparing the antigen content of the test serial to a reference preparation (Relative Potency) in accordance with the requirements specified in 9 CFR 113.8. Refer to Veterinary Services Memorandum 800.90 for additional information concerning relative potency assays and requirements for references.

III. GUIDELINES

A. *In Vitro* Relative Potency Tests

An *in vitro* relative potency test that compares the antigen content of a test serial to that of a reference preparation using a parallel line immunoassay or equivalent method acceptable to APHIS may be used to determine the antigen content of a serial of bovine rhinotracheitis vaccine.

B. Requirements for the Reference

The definition of a reference is contained in 9 CFR 101.5, and the requirements for references used in *in vitro* tests for serial release are specified in 9 CFR 113.8. Additional guidance is provided in Veterinary Services Memorandum 800.90.

1. Qualification of Final Product Master References

a. Master References that are non-frozen final product are qualified in animals directly using a minimum of 20 vaccinates and 10 controls in the host animal immunogenicity study.

b. Serum samples taken from animals in the reference qualification study that are stored frozen may be used in future reference requalification studies.

2. Qualification of Frozen Master References

a. Master References that consist of final product or purified antigen that is stored frozen are qualified indirectly in host animals using a Qualifying Serial. Qualifying Serials must be produced in accordance with the current filed Outline of Production and tested for immunogenicity in a manner acceptable to APHIS. Additional guidance on establishing a Qualifying Serial is provided in Veterinary Services Memorandum No. 800.90

b. The Qualifying Serial must be administered to a minimum of 20 vaccinates in the host animal immunogenicity study. Use 10 unvaccinated animals as controls.

c. Serum samples taken from animals in the reference qualification study that are stored frozen may be used in future reference requalification studies.

C. Working References

1. Master References that have been qualified in a manner acceptable to APHIS as specified in paragraph III.B. may be used throughout dating as the Working Reference in the serial release potency test. Additional guidance is provided in Veterinary Services Memorandum No. 800.90.

2. Qualifying Serials produced in accordance with the current filed Outline of Production and tested for immunogenicity in accordance with methods acceptable to APHIS may be used as the Working Reference in the serial release potency test. Additional guidance is provided in Veterinary Services Memorandum No. 800.90.

D. Expiration Date of the Reference

1. As specified in 9 CFR 113.8, the expiration date of the Master Reference shall be equal to the dating of the product or as supported by data acceptable to APHIS.

2. The expiration date of a Working Reference stored in a manner similar to a serial of licensed product shall be the same as the expiration date of a serial of the licensed product.

3. Expired references may not be used in serial release potency tests unless requalified.

E. Requalification of the Reference

References may be requalified by one of the following methods:

1. The host animal serological test prescribed in 9 CFR 113.216(c)(2) may be used to requalify an expired Master Reference for use as the Working Reference in the serial release potency test provided that:

a. The Master Reference is directly administered to test animals as non-frozen final product.

b. Master References that are frozen (or that are purified antigens) are indirectly administered to animals using a Qualifying Serial

c. Serum samples for titer determination are collected at the same time interval as was used in the original Master Reference qualification test.

d. The serum neutralization test used to titer serum samples collected during the Master Reference requalification study is the same *in vitro* method that was used to titer samples when the reference was qualified.

e. The serum antibody titers of test animals used in the qualification and requalification studies are statistically equivalent in side by side testing.

2. An expired Master Reference may be requalified by using in vitro methods to compare to a Reference Standard that has been prepared and validated under the auspices of a National Control Authority or a recognized Standards Organization acceptable to APHIS. Additional guidance is provided in Veterinary Services Memorandum No. 800.90.

3. The dating of a Working Reference that was established by testing a Qualifying serial in host animals may be extended by comparison to the Master Reference or by using the serial release potency test prescribed in 9 CFR 113.216(c)(2) to measure serological response in host animals. Additional guidance is provided in Veterinary Services Memorandum No. 800.90.

F. Test Interpretation

The results of each serial tested for potency using a parallel line assay or equivalent method acceptable to APHIS must be interpreted as specified in 9 CFR 113.8(b). To be eligible for release, the relative potency of each serial must be $ 1.0.

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